Results: Cartilage cultured in the presence of blood showed a decrease of proteoglycan synthesis rate of 70%, an increase of proteoglycan release of 100%, and a decrease of proteoglycan content of 15% after 16 days of culture (all $p<0.05$). This blood-induced damage of the cartilage matrix was limited by IL-4 in a clear dose-dependent way. Addition of 100 ng/ml IL-4 during blood-exposure reduced the proteoglycan synthesis rate with only 45%, and decreased the proteoglycan release with 30% compared to control (all $p<0.05$). Moreover, proteoglycan content was normalized. The combination of IL-4 and IL-10 was clearly more protective against damage caused by blood. This was especially evident for the proteoglycan synthesis which was completely normalized. Furthermore, treatment with a combination of the two cytokines was significantly better than the effect of IL-4 and IL-10 alone ($p<0.05$).
Conclusions: Besides IL-10, as shown previously, also IL-4 protects against blood-induced cartilage damage. The combination of these two cytokines is clearly the most protective. In addition to the direct effects on cartilage, both cytokines are synergistic in inhibition of inflammation. As such, this justifies further evaluation of the combination of IL-4 and IL-10 in prevention and treatment of blood-induced joint damage.

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BRADYKININ, THROUGH B_2 RECEPTORS, ACTIVATES THE RELEASE OF THE CYTOKINE INTERLEUKIN 6, THE CHEMOKINE INTERLEUKIN 8, AND THE METALLOPROTEINASE 3 IN HUMAN KNEE CHONDROCYTES

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Purpose: Bradykinin (BK, H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) is a proinflammatory and algogenic peptide: it releases inflammatory mediators and sensitizes sensory afferents through the activation of B_2 receptors expressed on the membrane of several cell types, including synoviocytes and chondrocytes. Aim of the current investigation was to investigate if BK and B_2 receptor can participate into mechanisms which are involved into ostearthritis (OA) degenerative events, and to evaluate the possibility to prevent them through B_2 receptor blockade, using the highly selective and potent B_2 receptor antagonist MEN16132 (m.w. 873.16).
Methods: Human chondrocytes (Lonza, CC-2550) were cultured in F12/DMEM 1:1 added with CGM Singlequots (Lonza, CC-4409) and Gln 2mM and used up to fifth passage. Experiments were performed with cells at confluence plated onto 24-well plates. Cells were incubated at the indicated concentration of BK in F12 medium supplemented with foetal bovine serum (1%), penicillin (50 μg/ml), streptomycin (50 μg/ml), amphotericin B (0.75 μg/ml), Gln (2 mM), and captopril (1 μM). At the end of the experiments, surnatants were collected and stored at -80°C and used for the dosage of interleukin 6 (IL-6), interleukin 8 (IL8), and metalloproteinase 3 (MMP3). IL-6, IL-8, and MMP3 contents in the supernatant were assayed by commercially available enzyme immunoassay kits (Promokine PK-EL-61606, PK-EL-61806, and Biosource KAC1541). Data are expressed as mean ± s.e.m. or 95% confidence limits in parentheses of 3 to 5 experiments, each in triplicate.
Results: Time-course experiments (2 - 96 h) indicated that BK (1 μM) induced a release of IL-6, IL-8, and MMP3 which increased over the time, stably peaked after 24 h of incubation, and remained constant up to 96 h. The maximal production of IL-6, IL-8 and MMP3 induced by BK (24 h incubation) was 557±39 pg/ml, 1267±169 pg/ml, and 5.52±0.73 ng/ml, respectively and was resembling that induced by the pleiotropyc cytokine TNFα (0.1 ng/ml)(817±350 pg/ml, 1778±198 pg/ml, and 1.96±0.2 ng/ml, respectively). Concentration-response curves to BK (0.1 nM - 1 μM, 24 h) indicated EC_{50} values of 10 nM (5.4-18.6) in inducing an augmented release of IL-6, 9.7 nM (3.7-32.5) for the release of IL-8, and 9.1 nM (1.1-7.6) for MMP3 secretion. This effect provoked by BK (100 nM, submaximal concentration) were concentration-dependently prevented by the pretreatment of HCCK (30 min) with the selective B_2 receptor antagonist MEN16132, and IC_{50} values were 1.7 nM (1.0-2.8) to inhibit IL-6 production, 2.2 nM (0.7-7.1) for IL-8, and 0.7 nM (0.3-1.4) for inhibition of MMP3 secretion.
Conclusions: These findings disclose novel actions of BK that imply its possible involvement in joint degenerative diseases, and indicate B_2 receptor blockade as a potential therapy in OA pathology.

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CARTILAGE THICKENING IN EARLY RADIOGRAPHIC KNEE OSTEOARTHRITIS - FACT OR ARTIFACT?

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Purpose: In animal studies of osteoarthritis (OA), increases in cartilage thickness have been observed at the early phases, due either to cartilage hypertrophy or edema. Evidence for cartilage thickening in human OA, however, remains enigmatic. Cross sectional analyses and one longitudinal study in human with MRI have indicated that medial osteophytes, in the absence of medial joint space narrowing (JSN), may be associated with cartilage thickening in the medial femorotibial compartment in human OA, particularly in the external aspect of the medial femoral condyle (ecMF). However, comparisons may have been confounded by differences in age, BMI and other factors.
To eliminate confounding by between-person differences and to enhance sensitivity, we performed a within-person between-knee analysis of cartilage thickness in knees with unilateral osteophytes and without JSN. The primary endpoint was the between-knee cartilage thickness differences in ecMF (in subjects with unilateral medial osteophytes), the secondary endpoint was thickness differences in the external lateral femur (in those with unilateral lateral osteophytes), and the exploratory endpoints were thickness differences in all other femorotibial subregions.
Methods: 83 OA Initiative participants were selected from 4800 cases (public use data sets 0.E.1 [imaging] and 0.2.2 [clinical]), who displayed definite osteophytes and no JSN in one knee and no signs of radiographic OA in the other knee, according to the site readings. The radiographs were reviewed by a central reader (F.R.) and 61 participants were included. Cartilage thickness was measured in four femorotibial cartilage plates (medial/lateral, femur/tibia) and in 16 femorotibial subregions, using sagittal DESSwe MR images and dedicated software (Chondrometrics). Within-person between-knee differences were calculated with a paired t-test and general linear models. No adjustment for multiple comparisons was performed.
Results: Of the 61 participants (age 60.8±9.6 yrs; 29m/32f; BMI 27.8±4.7) 48% displayed medial, 36% lateral, and 16% bicompartimental (mostly tibial) osteophytes. Knees with osteophytes had thicker cartilage than contralateral knees in the external medial (ecMF:+5.5%,p=0.02) and external lateral femur (ecLF:+4.1%,p=0.01), but in no other subregions. Knees with only medial osteophytes displayed greater thickening in ecMF (+7.8%) than ecLF (-0.1%), those with only lateral osteophytes greater thickening in ecLF (+5.0%) than in ecMF (+1.7%), and those with bicompartimental osteophytes similar thickening in both subregions (+17%/+15%, respectively). There was no significant effect of age, sex, or BMI on between-knee differences.
Conclusions: Knees with early radiographic OA (definite osteophytes, no JSN) displayed thicker cartilage than the contralateral knees without radiographic OA. The differences were specific to external medial femoral and external lateral femoral subregions and appeared to be locally mediated by (tibial) osteophytes in the same compartment.

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SUPEROXIDE DISMUTASE 2 DOWNREGULATION AND MITOCHONDRIA RESPIRATION IN OSTEOARTHRITIS

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Purpose: Oxidative phosphorylation takes place at the mitochondrial respiratory chain and is the major source for the production of ATP. A by-product of this respiration are reactive oxygen species (ROS). ROS are involved in signalling processes but when at high levels contribute to oxidative damage. The major ROS include superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). The mRNA and protein levels of the major inhibitor of superoxides in the mitochondria, superoxide dismutase 2 (SOD2), have been shown to be downregulated in osteoarthritic compared to healthy joint cartilage.
This study characterises the effects of reduced levels of SOD2 on chondro-

cyte mitochondria as well as the effect on signalling pathways leading to altered collagenase expression.
Methods: RNA intereference was used to characterise whether the reduction of SOD2 levels affects matrix metalloproteinase (MMP)-1 and MMP-13 gene expression along with superoxide levels produced by the mitochondria in human articular chondrocytes (HAC). For the MMP-1 and MMP-13 expression, cells were then serum starved for 24 hours and stimulated with IL-1. MMP-1 and MMP-13 mRNA levels were determined by real-time RT-PCR. To assess the levels of superoxide and SOD2 protein, HAC were stained with MitoSOX™ -Red, a specific indicator for superoxide in the mitochondria, or an anti-SOD2 antibody. To compare the mitochondrial respiratory activity between HAC from healthy patients (neck of femur fractures, NOF) and from osteoarthritic patients (OA), cells were cultured for 3 days after extraction from the cartilage and analysed for respiratory activity using the Oroboros Oxygraph-2k respirometry system.
Results: SOD2 depletion by RNA interference led to a significant decrease in basal MMP-1 mRNA expression and a significant reduction in the level of MMP-1 and MMP-induction following IL-1 stimulation. SOD2 depletion by RNA interference also led to a significant increase in mitochondrial superoxide levels.
In terms of respiratory capacity, OA chondrocytes have on average 65% higher respiratory chain capacity than NOF chondrocytes. However, although OA chondrocytes have a more active respiratory chain, it appears less efficient (25%) to that of HAC from NOF patients.
Conclusions: Depletion of SOD2 in OA chondrocytes leads to a significant decrease in the expression levels of the collagenases MMP-1 and MMP13, indicating that the decrease of SOD2 expression in OA cartilage may represent a chondroprotective mechanism. However, this depletion leads to a significant increase of mitochondrial superoxide levels. Combined with the increased levels of unutilised protons and electrons in the respiratory chain, these effects can potentially alter the mitochondrial membrane potential of the cells causing cellular dysfunction.

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GLATIRAMER ACETATE (GA), A PEPTIDE IMMUNOMODULATORY DRUG, INHIBITS INFLAMMATORY MEDIATORS, MMP-13 ACTIVITY AND COLLAGEN DEGRADATION IN OA CARTILAGE

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Purpose: Glatiramer acetate (GA), the generic name for Copaxone, is an immunomodulatory agent used in the treatment of multiple sclerosis, which has been to shown to induce interleukin-1 receptor antagonist (IL-1Ra) production in macrophages and microglial cells. In osteoarthritis, the production of inflammatory mediators, particularly IL-1 by chondrocytes, may be important in the pathogenesis and progression of OA. We therefore tested the effects of GA on the catabolic activities of chondrocytes in OA cartilage explants cultures.
Methods: Cartilage slices were obtained from the knees of patients with advanced OA and undergoing knee replacement surgery. Non-arthritic knee cartilages were obtained from autopsy patients within 24h (NDRI, Philadelphia). Matrix metalloproteinases proMMP-13 ELISA kits were from R&D Systems, (Minneapolis, MN). Predesigned TaqMan PCR primers were purchased from Applied Biosystems (CA).
Results: We have previously shown that OA cartilage explant cultures spontaneously release inflammatory mediators such as nitric oxide, Prostaglandin E2 (PGE2), interleukins including IL-1betaand matrix metalloproteinases (MMPs). In this study we evaluated the chondroprotective property of GA in monolayer cultures of primary OA chondrocytes and cartilage explant cultures. GA (5-100ug/ml) treatment dose dependently increased transcription (QPCR) and production (ELISA) of sIL-1Ra ($p>0.001$) in normal and OA Chondrocytes. GA also intrinsically increased ($p<0.001$) sIL-1Ra production by OA cartilage explant cultures. Furthermore, addition of GA: 1) inhibited both spontaneous and IL-1 induced inflammatory mediators such as nitric oxide (NO) (9.0+1.5 to 1.5+0.5 uM; $p<0.01$) and PGE2 (100.6+18.5 to 15.1+6.1 ng/ml; $p<0.01$) production; 2) inhibited spontaneous and IL-1 induced proMMP-13 secretion (200.6+90.5 to 78.5+15.1ng/ml; $p<0.001$); 3) inhibited APMA activated total MMP-13 activity by more than 30-50% ($p<0.01$); 4) inhibited collagen degradation as assayed by CTXII ELISA (17.4+1.7 to 4.5+1.9 ng/ml; $p<0.01$). All these chondroprotective effects of GA were dose dependent and significance was reached between 10-100 ug/ml.
Conclusion: Glatiramer acetate is a complex heterogeneous mixture of polypeptides that exhibits "chondroprotective" properties in OA cartilage, inhibiting the production of inflammatory mediators as well as MMP-13 expression/activation. The data suggest that these effects may be due to upregulation of IL-1Ra. Based on these studies, we propose that GA may have potential for disease modifying properties in OA and should be evaluated in vivo animal studies.

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ADIPONECTIN AND LEPTIN EXHIBIT DIFFERENT PATTERN OF PRODUCTION IN CARTILAGE FROM PATIENTS WITH OSTEOARTHRITIS

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Purpose. Based on the association between obesity and osteoarthritis (OA), increasing studies aimed to determine the contribution of adipokines in OA. As adiponectin and leptin may exhibit opposite inflammatory effects and display different patterns of distribution between the joint and the circulating compartment, we compared the production of both adipokines in cartilage from human OA-affected joints in relation with the grade of cartilage destruction and with the Body Mass Index (BMI) of the patients.
Methods. The production of leptin and adiponectin was determined by ELISA in conditioned media from cultured full-depth cartilage biopsies obtained from patients with OA. The severity of OA cartilage lesion was then evaluated after histological analysis of each specimen and was graded using the Mankin score.
Results. The results indicated that the production of adiponectin in OA cartilage are quite different from that of leptin. A positive association has been found between the BMI of the patients and the production level of leptin while the synthesis of adiponectin did not change with the BMI. In addition, a grade-dependent increase in the production level of leptin was shown for non obese patients ($BMI<30kg/m^2$). The synthesis of leptin strongly increased in obese patients ($BMI>30kg/m^2$) between the low and the moderate OA grades, but did not change anymore for the most severe OA grade. The adiponectin production was slightly elevated in cartilage samples with moderate or advanced OA compared to specimens with low histological OA score, but the difference did reach statistical significance.
Conclusion. These findings indicated that leptin and adiponectin exhibit different pattern of production in OA cartilage. The leptin level is strongly associated with both the grade of cartilage destruction and the BMI of the patients. Conversely, the production of adiponectin is slightly up-regulated in damaged cartilage independently of the OA score and the BMI of the patients.

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WNT3A MODULATES CHONDROCYTE PHENOTYPE THROUGH ACTIVATION OF BOTH CANONICAL AND NON-CANONICAL PATHWAYS

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Purpose: Injury to the articular cartilage results in the activation of Wnt signalling which may represent a homeostatic mechanism. Indeed disruption of Wnt signalling results in osteoarthritis both in humans and experimental models. Wnt ligands are traditionally categorized based on their capacity to activate either the β-catenin-dependent canonical pathway or any of the non canonical pathways including the Ca^{2+}/Calmodulin-dependent kinase II (CaMKII)-dependent or the PKA-dependent pathways, the planar cell polarity pathway and the Wnt/ROR-mediated pathway. The clear-cut separation between canonical and non-canonical Wnts has been recently challenged and therefore the purpose of the study was to investigate through which pathway Wnt3a modulates the cartilage phenotype and regulates cartilage homeostasis in human articular chondrocytes.
Methods: Primary adult human articular chondrocytes were isolated from preserved areas of the cartilage of patients undergoing knee arthroplasty for osteoarthritis. Detection of protein and protein phosphorylation upon Wnt3a stimulation was evaluated by western blotting. Intracellular calcium mobilization was detected by cellular accumulation of FURA-4 dye. The activation of the canonical pathway upon Wnt3a stimulation was evaluated